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Studies on incidence and antibiotic susceptibility pattern of bacterial pathogens in dairy products

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Abstract
Milk and milk products are ideal foods for all age groups in both rural and urban people all around the world. This study reports the microbial prevalence in milk and milk products and antibiotic susceptibility pattern of *E. coli*, *S. aureus*, *S. typhi* and *B. subtilis* isolated from raw milk and different dairy products. Out of 300 samples of raw milk, cream, curd, paneer (75 each), 153 isolates were found to be positive in which higher rate of incidence was found in raw milk 58 (77.33%), cream 24 (32%), curd 33 (44%), and paneer 38 (50.66%). The antibiotic susceptibility pattern was also studied for the isolated organisms which provide a varying degree of response against the isolated pathogens which provides a varying degree of response against the isolated pathogens. Six antibiotics used were mostly found sensitive to organisms. Amoxicillin were found resistant to *E. coli* and *S. typhi*. Hygienic conditions should be maintained to reduce the incidence of such pathogens in milk and milk products while the data of antibiotic susceptibility pattern provides the social help to maintain the public health. Results clearly suggest a possibility of potential public health threat of pathogens isolated from the contamination of milk and milk products mainly due to unhygienic processing and environment.

Keywords*:* Antibiotic Susceptibility, milk, pathogens, incidence.

1. Introduction
Raw milk represents an ultimate growth medium for microorganisms. The occurrence of both small and large scale outbreaks of illness attributed to milk borne (Ekici *et al*., 2004) [4]. Milk is highly nutritious food ideal for microbial growth and the fresh milk easily deteriorates to become unsuitable for processing and human consumption (FAO, 2001) [6]. One of the major factors for the public health concern in milk and milk products is the presence of pathogenic bacteria [5]. Over the last few years food poisoning and food safety has become very topical subject, eliciting a great deal of public concern to many people all over the world. This is a result of rising food borne pathogens that continue to cause outbreaks of food borne diseases caused by eating food contaminated with pathogenic microorganisms or their products [25]. A broad spectrum of microbial pathogens contaminates human food and water supplies and cause illness after they or their toxins are consumed. These include a variety of enteric bacteria, aerobes and anaerobes, viral pathogens and yeasts. During past decades microorganisms such as staphylococcus spp. *Salmonella* spp. were reported as the most common food borne pathogens that are present in many foods and able to survive in milk and fermented milk products [17]. Milk treatment and processing can either inhibit or support the microbial multiplication [2]. The food borne pathogens that are able to survive and thrive in post-pasteurization environments can lead to recontamination of dairy products. These pathways pose a risk to consumers [15]. So the presence of knowledge about the current scenario about the antibiotic resistance of different microbes isolated from milk and dairy products are less in number. Hence the present study was framed to study the antibiotic susceptibility pattern of isolated microbes from the milk and dairy products.

2. Material and Methods
2.1. Collection of samples and isolation of different organisms
A total of 300 samples of milk and other dairy products were collected from different areas and isolation of *E. coli*, *S. typhi*, *S. aureus* and *B. subtilis* were done on the different selective Medias (MacConkey Agar, Baird's Parker Agar, Salmonella- Shigella Agar, Trypticase Soy Agar). Identification and confirmation of isolated microorganism was done on the basis of

cultural morphological and biochemical characteristics given in Bergey's Manual of Systematic Bacteriology [10].

2.2. Antibiotics used
All the antibiotics were purchase from Hi-Media Laboratory Pvt. Ltd., Mumbai. Gentamycin (Gen) 30 mcg/disc, Tetracycline (Tetra) 10 mcg/disc, Kanamycin (Kana) 30 mcg/disc, Ciprofloxacin (Cipro) 5 mcg/disc, Ofloxacin (Oflox) 5 mcg/disc and Amoxycillin (Amox) 30 mcg/disc.

2.3. Reviving of culture
The culture isolated were sub cultured on Nutrient Agar media before antibiotic susceptibility testing. It is done by streaking the cultures on Nutrient Agar media plates and incubation was done for 24 hrs at 37 °C. This prepares the culture in a pure form for the further tests.

2.4. Antibiotic susceptibility testing
The isolates were tested for their susceptibility to six different antimicrobial drugs: Gentamycin (Gen) 30 mcg/disc, Tetracycline (Tetra) 10 mcg/disc, Kanamycin (Kana) 30 mcg/disc, Ciprofloxacin (Cipro) 5 mcg/disc, Ofloxacin (Oflox) 5 mcg/disc and Amoxycillin (Amox) 30 mcg/disc.
The isolates were subjected to antibiotic susceptibility test as per protocols of Kirby and Bauer (1966). Bacterial lawn culture was prepared on Muller Hinton agar by swabbing overnight broth culture. Standard antibiotic discs (antibiotic concentration in µg) were impregnated on the surface of the agar plates. The plates were then incubated at 37 °C for 24-48 hrs. The zone of inhibition were measured and recorded in mm diameter and interpreted as susceptible or resistant as per CLSI (2009), (Normanno *et al*., 2006).

3. Results
3.1. Isolation of different organisms
A total of 300 milk and different dairy products were studied out of which 153 (51%) were found to be positive for the pathogenic bacteria. Out of 153 isolates 72 were confirmed as *E. coli,* 15 *S. typhi,* 43 *S. aureus,* and 23 *B. subtilis.* A higher incidence showed in the raw milk (77.33%) in compared to paneer (50.6%), curd (44%) and cream (32%) (Table1).

Table 1: Incidence of bacterial pathogens in Milk and Milk Products

S. No.	Sample Type	Sample Size (n = 300)	No. of isolates	*E. coli*	*S. typhi*	*S. aureus*	*B. subtilis*
1.	Raw Milk	75	58 (77.33%)	22 (37.93%)	9 (15.51%)	18 (31.03%)	9 (15.51%)
2.	Cream	75	24 (32%)	9 (37.5%)	0	11 (45.83%)	4 (16.66%)
3.	Curd	75	33 (44%)	19 (57.5%)	3 (9.09%)	6 (18.18%)	5 (15.15%)
4.	Paneer	75	38 (50.66%)	22 (57.89%)	3 (7.89%)	8 (21.05%)	5 (13.15%)

The isolated pathogens were found to possess a high level of antibiotic susceptibility towards the different class antibiotics tested.

3.2. Antimicrobial activity of different antibiotics against isolated microorganisms
Disc diffusion method
A total of six antibiotics were chosen to determine the antibiotic susceptibility pattern against the four isolated pathogens that is *E. coli*, *S. aureus*, *S. typhi*, *Bacillus subtilis* (Table 2).

Table 2: Zone of inhibition different antibiotics using disc diffusion method

S. No.	Antibiotics	Disc Diffusion Method (Zone of inhibition in mm)			
		Microorganisms			
		E. coli	*S. aureus*	*S. typhi*	*B. subtilis*
1	Gentamycin	20 mm	32 mm	22 mm	26 mm
2	Tetracycline	14 mm	28 mm	20 mm	25 mm
3	Ciprofloxacin	26 mm	20 mm	24 mm	26 mm
4	Kanamycin	15 mm	25 mm	14 mm	22 mm
5	Ofloxacin	24 mm	30 mm	16 mm	30 mm
6	Amoxycillin	12 mm	35 mm	12 mm	22 mm

Zone of inhibition was compared with CLSI chart for the isolated pathogens. In the results *E. coli* showed sensitive result for Gentamycin, Ciprofloxacin and Ofloxacin, intermediate result for Tetracycline and Kanamycin and also found to be resistant against the Amoxcycillin. The present result showed that the *E. coli* were most sensitive against ciprofloxacin with the zone of inhibition of 26mm. Similarly for the *S. aureus* they were found to be sensitive for Gentamycin, Tetracycline, Ofloxacin and Amoxcycillin, and intermediate result with Ciprofloxacin. In the present study it was found that *S. aureus* were most sensitive for Amoxcycillin with the zone of inhibition as 40 mm. *S. typhi* was seen sensitive against Gentamycin, Tetracycline, Ciprofloxacin and was the intermediate result for kanamycin and Ofloxacin, it was also resistant with Amoxcycillin. *Bacillus subtilis* was found to be sensitive against all the studied antibiotics with the highest zone of inhibition of 30 mm against Ofloxacin. The present study revealed that the isolated pathogenic microbes were mostly sensitive to the studied antibiotics.

4. Discussion
The milk and different dairy products evaluated during the present course of investigation revealed the presence of different pathogens viz. *E. coli*, *S. typhi*, *S. aureus* and B. Subtilis. These findings can be corroborates with the previous findings in which the similar incidence for *E. coli*, *S. aureus* and *S. typhi* was observed by Ekici *et al.,* (2004) [4]. Oksuz *et al.,* (2003) [3] reported *E. coli* 0157:H7 at the rate of 1% in 100 samples of raw milk. Soormo *et al.,* (2002) [20] also isolated 57% *E. coli* in raw milk samples.
The present study was also similar in comparison to the findings of Gwida and Gohary (2013) [7] showing the incidence of 56.66% *S. aureus* and 36.66% *E. coli* in raw milk. Similar incidence for *E. coli*, *S. aureus* and *S. typhi* was recorded by Zagare *et al.,* (2012) [25]. Reports of Kumar and Prasad (2010) [11], Hassan and Afify (2007) [9] and Singh and Prakash (2008) [19] also reported the same findings. Mary *et al.,* (1992) [12] and Oranusi *et al.,* (2007) [16] states that *E. coli*, *S. aureus* and *L. monocytogenes* occur frequently in milk products such as curd and cottage cheese. The incidence of spices of *E. coli* itself in milk and milk products as a possible cause of food borne disease, is not significant if *E. coli* is normally ubiquitous organism (Hahn 1996) [8] yet the pathogenic strain if present is harmful to the consumers. The presence of bacteria like *E. coli*, *S. aureus*, *S. typhi* in milk

suggested contamination from various sources such as animal tests, udder etc, unclean hands of workers, environmental dust and microbes unclean utensils in and other [25]. This finding supports the present study.

Salmonella typhi in milk and milk products cause typhoid fever. Various internal organs such as spleen and liver becomes infected by *salmonella spp.* presence of *salmonella spp.* indicate contamination due to water used in dairy farm. The isolation of coliform and other food pathogens from these dairy products pose a serious threat to food safety, especially, locally processed foods which are consumed without further processing, great attention should therefore be given to the microbiological safety of these products because their direct consumption may cause health hazard to the consumer [18].

In the present study varying degree of resistance pattern towards different antibiotics was observed. Amoxycillin resistance was seen against the *E. coli* and *S. typhi* in the present study, whereas the isolated pathogenic microbes were susceptible to the selected antibiotics. Afroz *et al.,* (2013) [1] also studied the antibiotic susceptibility pattern for the isolated *E. coli* and *S. aureus* isolated from the cream powder milk sold in the market area of Dhaka, Bangladesh. Similarly Thaker *et al.,* (2013) [21, 24] also conducted the isolation and identification of *S. aureus* from the milk and milk products and drug resistance pattern in Anand Gujrat, which is similar to the present study, but they investigated the resistance of Gentamycin (10.00%) against the *S. aureus* which is in contrast to the present study. The study of Virpari *et al.,* (2013) [24] studied the antibiotic sensitivity pattern of *E. coli* isolated from milk and dairy products which is comparable to the present study.

The variation in the antibiotic susceptibility pattern was observed due to incubation temperature and duration, media used for making dilutions, amount of inoculums used and difference in the species isolated.

5. Conclusion

The majority of raw milk and various milk products samples were found contaminated which requires strict management for effective measures for hygienic and sanitary practices.

The indiscriminate use of antibiotic for the prophylactic as well as other therapeutic purpose could be the reason for increased antimicrobial resistance. So more studies are required to combact such problems and to make data available for the antibiotic susceptibility pattern of prevailing pathogens. This could help the local society and strict measures could be taken to improve the human health respectively.

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